

Dehydrogenase Media.

126.

2/18/48.

Make up $\frac{1}{2}$ % La (L-asamino acids) in .2% w/v Ammon. Molybdate. + tryptone
A. - B. Add 2% Sodium Succinate.

After autoclaving, A is blue; B is lt. yellow.

K-12. N.G. on A. Colorless on B.

W-236 x Y40. On EMS.

G. 5-10% lac- Therefore W-236 is not lac₁+. Call the gene "reverting" in W108-W117 sl₁+ (Suppressor of lac₃). Call the differential between Y40 and W-236 sl₂+. If sl₁+ ≠ sl₂+ then some of the lac+ recombinants will be glu- and v.v.
Empirical on lac + Glus.:

- (A) { 9 cultures were lac- (\pm ?) but glucose +.
10 cultures were lac+ glu+
- (C) 23 cultures were lac- glu-
- (B)

Streak out samples of each type on lac + Glu EMB.

		Lac	Glucose
1	L- / / +	++	-
2	L+ / / -	++	++
3	L- / / -	-	-
4.	L+ / / -	-	-

This type suggests that the mutations differentiating W117 from W236 is at distinct loci from the one between W108 and W117

A-W108 X

A

~~B-1453 X~~

B

1. W239 ++

2. W243 ++. Also + and - (as covers plate. ± not weighed)

3. W245. ++

Streak out parents.

108 R/S varieties, 1± / > 200 + recessive in streaks.

239 < 5% mottled. Note colony - darkening around +
243 all - OK. Thin colonies.

245 50% mottled.

Crosses inconclusive!, etc.

243?

Y10 80 secs. Watson's Sterilamp.

70 plates \times 200 cols. = 14,000 scored.

Very few entirely - found.

	++ and -	254.
	++ and -	255
0	- (and++)	256

Also, 9 cultures recovered which are not = but ± :

24h. ♂ Pick 2 for study: 257
258

58-161 80 sec. Water's sterilizer

50 plates \times 200 = 10,000 secord.

- W-253

About 10 others picked were not mutant. Pick to glucosate broth.

Lac Huttonian Run.

February 21, 1948.

Y/O 80 sees. Watson's Lamps ETPB.
 100 plates x ca. 1500 cols. = 150,000 (very rough est'n) colonies

68 mutants $\frac{1}{1200}$ total 14 subord.

		Glu	lac	gal	Slow lac	Mgal.
W-:	259	v. slow	-	-	-	-
	260	-	-	-	108	+
	261	-	-	-	108	-
	262	++	-	-	Lac	+
	263	-	-	-	Lac	+
	264	-	-	-	Lac	+
	265	-	-	-	Lac	+
	266	-	-	-	Slow	+
	267	-	-	-	lac	+
	268	-	-	-	108	+
	269	-	-	-	108	-
	270	-	-	-	108	+
	271	-	-	-	Lac	+
	272	-	-	-	Lac	+
	273	-	-	-	Lac	+
	274	-	-	-	Lac	+
	275	-	-	-	Lac	+
	276	-	-	-	Lac	+
	277	-	-	-	108	-
	278	-	-	-	Lac	+
	279	-	-	-	-	-
	280	+ diffuse.	-	-	108	+
	281	-	-	-	lac	+
	282	v.	-	-	(145)	-
	283	-	-	-	Lac	-
	284	-	-	-	108	+
	285	-	-	-	108	-
	286	-	-	-	Lac	+
	287	-	-	-	Lac	+
	288	-	-	-	Lac	+
	289	-	-	-	Lac	+
	290	-	-	-	Lac	+
	291	-	-	-	Lac	+
	292	-	-	-	108	-
	293	-	-	-	Lac	+
	294	-	-	-	Lac	+
	295	-	-	-	Lac	+
	296	-	-	-	Lac	+
	297	-	-	-	108	-
	298	-	-	-	108	+
	299	-	-	-	(145)	-
	300	-	-	-	Lac	+
	301	-	-	-	108	-
	302	-	-	-	Lac	+
	303	-	-	-	Lac	+
	304	-	-	-	Lac	+
	305	-	-	-	Slow	+
	306	-	-	-	Mel-lac	-
	307	-	-	-	108	+

Non-saccharol Malt. Cact.

	Sucrose	Maltose	Lactose	Galactose	Glucuronic	Hegel
308	-	-	-	+	108	+
309	+	+	-	+	Lac	+
310	+	+	-	+	Lac	+
311	+	+	-	+	Lac	+
312	-	-	-	+	108	+
313	+	+	-	+	Lac	+
314	+	+	-	+	Lac	+
315	+	+	-	+	Lac	-
316	+	+	-	+	Lac	-
317	+	+	-	+	Lac	-
318	+	+	-	+	Lac	+
319
320
321
322
323
324
325
248
248	1	+	-	+	Lac	+
249	2	-	+	+	108	+
250	3	+	+	+	Lac	-
253	4	-	+	-	Glucose	++
254	5	+	+	+	Gal	-
255	6	+	+	+	Gal	-
256	7	+	+	+	Gal	-
257	8	-	-	±	108	-
258	9	-	-	±	108	+
259.	10	+	+	+	Lac slow.	+
<i>S. parac A</i>					inhibited	
<i>lambdas 27</i>					-	
<i>dublin 37</i>					-	
<i>E. coli ML</i>						

lac Mutations Recd.
Spontaneous control.

133

February 23.

Dil. Y10 suspension resed in 132 to 5×10^{-6} . Use 1 drop
(= .05 cc) per lac EMB plates. 20 plates.
ca. 800/plate = 16,000 Test all suspicious cultures.

10 examined. No mutants.

Cross - Test Lac, + Lac_Y
 February 24, 1948. EHS-Lac 8 plates each.

A. Y53 ×	Y87.	0/400 0/400 0/400 0/200 0/400 0/400	0/2600. B+H+L+
Lac _Y -	B-11-T ⁺		
(M ⁺) T ⁺			
B. Y53 ×	W67	'/300, °/200, °/300 °/300 °/200 °/200 °/200 °/200	2/2000
Lac _Y -	Lac _Y -		
C. W128 × Y87	Lac _Y -	°/300 °/400 °/200 °/200 °/400 °/400 °/200 °/200	0/2400
Lac _Y -			
D. W128 × W67	Lac _Y -	400 0/200, 0/100, 0/200, 0/100 0/1300 0/200 0/200 0/200 0/100	
Lac _Y -			
E. W120 × Y87	Lac _Y -	0/400, °/300, 0/400 0/500, 0/500 °/300 2/300 0/500	3/3200
Lac _Y -			
F. W120 × W67	Lac _Y -	°/400, 0/400, 0/100, 0/200 0/2000 0/300 °/100 0/100 0/200	
Lac _Y -			

Parents:			
Y53	°/2000. +.	Lac _Y .	Y53, Y87 (W128)
W67	"		
Y87	"	Lac _Y	W67, W120, (W128).
W120	"		
W128	"		

W128 may be a deficiency for both loci, or a double mutant. Dose heavily into T(m) + Megal!

February 23.

Spot checks on 1% Methyl EMB. 50/plate.

1 W-45	-
2 W-35	-
3 55 a	?
4 55 b	2
5 55 c	2
6 122	2 -
7 124	+
8 127	+
9 131	-
10 132	-
11 134	-
12 135	-
13 136	+
14 137	-
15 138	-
16 139	+
17 140	-
18 141	-
19 # 143	-
20 144	±

W-190 series.

21 190	+	38	218	+
22 192	+	39	219	-
23 193	+	40	222	-
24 194	-	41	223	+
25 196	+	42	225	+
26 197	+	43	228	+
27 201	+			
28 202	-			
29 205	+			
30 206	+			
31 208	-			
32 209	+			
33 211	+			
34 212	-			
35 214	-			
36 216	-			
37 217	-			

Check Stocks.

Feb. 28, 1948

Stocked out NA stocks on glu + Lac EMB:

	bac	Glu.
W- 108	ell-, popillating	
188	++ 1+1/1000 - (pop.)	- pop.
239	colonies, smooth, ±	v. small colonies. (beads?)
243	++	all ±
245	++ large +, small -	ell-, 2 colony eyes
251	+++	- glossy.
252	++ rough	all - rough
327	++ rough.	all -

253: slow + on glucose, may cut for lactose response. pH effect??
 Reproducing v. strongly on glucose

Specific Reactions

February 24 ff. 1948

1. W-35. Recombinate 55-a + 55-b; R test on lactose, Megal.

55a	(\ominus)	Relabel	W-
55b	(\ominus)	"	W-

2. Test W-353 papillae from glu, gal + mal on all three media \neq T1.
 5 + on all. T1 - sensitive.

3. Mix heavily into T(m) + .05% sugar

Box P14.	Lac	Mal	Megal	Rosa.	Sucrose.
24h.	W-45		<u>-</u>		
48h.			<u>-</u>		

P29.

W-145	<u>++</u>	<u>++</u>	<u>++</u>	++	+ + + + + + chitin Lac, Mal.
	all-	all-	all-		

W-243	<u>-</u>	<u>++</u>	<u>-</u>	<u>++</u>	---
	Mostly +	Mostly +	Mostly +		

W-125	<u>++</u>	<u>++</u>	<u>++</u>	<u>++</u>	
	all + chitin	Megal	all + chitin	Megal.	
W-128	=	=	=	=	

4. Test W45/Lac papillae on Megal. 9+. 0-

a) 37 125 Lac^{+R}. Test on Megal. All +.
 16 Megal^{+R} tested on Lac All +.
 1 128 Lact^{+R} " " Megal +.

33	W-145	Dnat ^{+R}	on Lac	All +
29	"	"	" Mal	" +

15	W-188	Glu ^{+R}	on Lac	All +
42	W-243	Lac+	on glu	All +
53	"	Mal+	on glu	All +.

Test w. 120 papillae on Megal. Megal - streaked on back.

6 all-
(apparently) ————— 2 + and - . Test ++ on Megal.
Both are Mg + .

No specific reversions noted.

Nitrogen sources

137.

Febr. 25, 1948.

Prepare, -N, perl:

glucose	1
NaCl	5
MgSO ₄	.1
K ₂ HPO ₄	3
KH ₂ PO ₄	1

and autoclave 50/125 flasks.

Add K-12 dil. suspension into:

P25.	A.	-	P27.	P29
B.	NH ₄ Cl 5% (.2%)	2.0 cc	v. dense +++	+++
C.	Urea 20%, stir filt. (.2%)	.5 cc	±	±
D.	Glycine, 15% (.5%)	1.5 cc	++	++
E.	Asparagine, 5%. (.2%)	2.0 cc	++±	++++! dense!

Final addition is ca. N/15 N.

This medium seems to be satisfactory for urease plating.

Cross - Test Lac Mutants.

~~#24~~

138

Reversins.

February 27, 1948.

~~+~~ x W-45 B. x Y-87

- (1) Test c W-45 for Lac-2, the bangal - set of the current lac - series.
3 plates each.

276
283
286
287
313
316
317

+++ ✓✓
++- -
+++ ✓✓
+++ ✓✓
+++ ✓✓
+++ ✓✓
+++ ✓✓

None of these are lac₂-.



- (2) Test 327 + 329 with Y10 + W236 on lac + Mel for suppression.

327 x Y10 on mature Synthetic. 5 plates. No colonies!
lac EMB 1 plate No colonies.

This is not a good test for lac + because it is not lac +.

- (3) 329 x 236. (W-35 lac + Rev. Hg- x W108 Sl. + lac₃-).
Many ++, --.



329 x Y10 on lac. Apparently all +.

5 x 500 = 2500 colonies tested. Therefore, there are at least

3 alleles at the lac₃₅ locus: +, -, and a = lac + bang -.

[Test 329 for mutation to +, and W-35 for relative frequencies of]
mutation to other states.

→ Test prototrophs on Hg S. 71 all +! Should have been ca. 8% -.

B. globigii

138a

Feb. 25, 1948.

"Constant" yellow strain from PW Wilson.

Preliminary irradiation: 1 drop broth culture / ~~Niger~~ GA plate.

40 secs. T

80 secs. T

120 secs ca 1000

108 x 243, 188.

139

Feb. 25, 1948.

'On glucose-EMS'.

w-108 x w-188.

Yield very low.

0/5. 0/5 0/16 Total : 0 / 41.
0/9 0/6.

w-108 x w-243.

0/13 0/7 0/10 ①/13 ① / 50.
0/7.

The + recovered might be a reversion. Cross should be repeated on a large scale.

Test Reversants of Lac -

140

March 1, 1948.

Cross with Y40: 4 plates each. Lac S.

A^3 w-235. Two classes noted: ++ and ±. (Allele?)

p3. All ++. -: 0/200, 200, 200, 200. ~~Streak at +/+ and parental~~

w-233 -: 0/300 0/200 0/400 0/400. 1? Streakout.

w-232. 0/200, 0/300 0/300 0/300.

w-234. 0, but hold. 0/1000 —

w-231 0/200 1/200 0/200. 2? S.O.

w-33. 0/150 0/150 0/200 0/100 ✓

w-34. 0/1000, 0/300 0/300.

w-327 x ~~Lac~~. Y40.

Lac S: 17+ : > 100 -. .

$T-L-L_3-B+M+$ $\times T+L+L_3+B-M-$

~~T-L-L₃-B+M+ Lac~~

∴ L_3 is linked to BM ~~Lac~~

Mal S: 7 plates: ca 300/plate. Some probable Mal- noted.

Streakout. ✓ 9 Mal- recovered. Test these on glucose. All -.

Cross W-45 x W-34 on Lac, Mal's + Leucine or + Threonine.

Lac : leucine. Very few lac - Only one recovered.

threonine ca 5% lac - Only four lac - recovered

Studied on EMB Lac + test purified ~~clones~~ clones. 1:

	T(0)	(B ₁)	(TnL)(TnL)B ₁
2:	-	+	-
3:	-	+	-
4:	-	+	-
5:	-	++	±

Mal Mostly - L. agar much clearer than ~~T~~ T.

Test some + and - on lac EMB. Find Rec. 7 found.

Threonine:

Mal - 5 / 32 are lac -

Purify the lac - 's.

Mal + 2 / 16 are lac -

		T(0)	B ₁	T	TB ₁	
M+	1	-	+	-	+	
M+	2	-	+	-	+	
M-	3	-	+	-	+	
M-	4	-	+	-	+	
M-	5	-	+	-	+	
M-	6	-	±	-	++	W6.8. 141-6
	7	-	+	-	+	

See over

Test more Thiomine Mal- segregants:

All Mal-.

Lac+	Lac-	? (Hartley -)
46	8	
42	5	3
59	4	4
50	3	8
42	7	4
	4	

Streak out prob. Lac- and test mutations.

O B_1 T TB_1

1. B_1
 2. B_1
 3. B_1
 4. TB_1 - - ± ++

5. B_1

6. B_1

7. B_1

8. B_1

9. B_1

10. B_1

11. B_1

12. B_1

13. B_1

14. B_1

15. TB_1

16. B_1

17. B_1

18. B_1

19. B_1

20. B_1

21. TB_1

22. TB_1

23. B_1

24. —

25. B_1

26. B_1
 27. B_1
 28. B_1
 29. B_1
 30. B_1

~~YY~~
~~BB~~

2/28 TB_1
 1/28 Protrate.
 25/28 B_1 -

W-339

W-337

{ -6
-6.

20. B_1
 21. TB_1

22. TB_1

23. B_1

24. —

25. B_1

March 1, 1948.

Strains out on Mal, Lac EMB. P29.

3/2/48. Lac-, rather alloallergenic. No papillae

Mal- (faint slow purple); Numerous papillae. Test on
Lac + Mal.

Papillae are alloallergenic. ~~Still~~ Still all Lac - Mal -.
(11) (8)

3/3/48. W-306 x 58-161 on Lac' S

3/4/48. Papillae noted in 306/L. Picks to Mal to check specificity.
All seem to be Mal- or Mal±. Strains out on Lac.

Test purified Lac^R on Mal.

W-306

142a

March 3, 1948.

W-306 x ~~58-161~~ 58-161 on Lac^y's.

5 plates.

Lac+	Lac-
11	14
9	8
9	17
4	8
4	10
37	57
	94

T-L+ B-M- ~~Lac^y~~ x T-L-B+M+Lac-

ca. near lac.

Test lac+, lac- n Mal.lac+: ~~1~~ Mal++ 29 Mal-test S. dysenteriae.

1. Mal- Lac+
2. Mal- Lac+
3. Mal+ Lac+
4. Mal+ Lac+

142-a6. Lac- 2 Mal++ 27 Mal-.

Test S. dysenteriae

1. Mal+ Lac-
2. Mal+ Lac-
3. Mal- Lac-
4. Mal- Lac-

∴ W-306 is a double mutant, Mal_x-Lac_y-.Kupf Lac^R (Lac+Mal-) as UV-

~~81 plates~~. March 2, 1978.

1 drop 10^{-5} dil. Y10/YB culture spread on each of 81 plates.
ca > 1500/plate. About 150,000 colonies scoreable
some plates > 2000.

9 suspicious colonies streaked out. All intact.

5 mutants recovered.

w- 331

w- 332

w- 333

w- 334

w- 335

[Compare with 68 mutants recovered from about the same number of colonies in Exp. 132].

Antisera to C1 + C2.

144

March 3, 1948.

Innunige rabbits against Y105 + Y109.

Purify antigens from broth cultures, wash in H₂O. Estimate cells.

386 F6 Y105. 10^9
 $\frac{3}{3}$

387 F6 Y105 10^9

383 F5 Y109 10^9

385 F3 Y109 2×10^9

All rabbits died in 12-20 hours. No post.

Fermentation tests.

145.

3/4/48.

EMB:

W-108 Y10 W-118 W-119

Mannose
1%

- +++ +++ * +++ *

Sorbose 1%

- - - -

v. poor growth (why?)
108 showed best growth.

Glucose 1%.

- ++ + + +++.

* indistinguishable.
Compare Marmal.

Melibiose
3%

K-12 Y10 W-306 W-55

- - - -

No detectable utilization

3/11/48.

Ethyline glycol- β -glucoside (any more?)

18h.
K-12
W-55
W-108
W-145
W-~~161~~
W-327
W-328

+ weeks
++
-
+
+++
-
-

K-12 W-55 W-145 W-108 Y53 327

Methyl β -L-acetylgalactoside.

-

Methyl α -D-xylopyranoside

-

Methyl β -D-xylopyranoside

-

Ethyline Glycol- β -D-glucoside

± ++

Melibiose.

±

- ± -

328+!

read me ambiguous!

3/3/48.

See 137 for "N" medium. Add K-12 lightly or Y10 heavily
into: 03.

	Bac.	A.S.	A.T.	
N(B ₁₂) _{100g}	1. Y10	+	-	
"	2. Y10	+	+	+
"	3. Y10	+	+	+
" - 4. K-12	-	+	+	N from amino acids!
N(0).	5 K-12	-	-	
N(urea)	6 K-12	-	±	
Urea + NH ₄ Cl.	7. K-12	-	+++	+++
NH ₄ Cl.	8. K-12	-	+++.	Urea not inhibitory.

In following, omit glucose; add NH₄Cl. for C-utilizers: ~~K-12~~ + TBS. (Y10).

P8

11.	--	-	-	-
12. glucose ^{1%}	-	+++	++++	✓
13. glycine	-	-	-	-
14. asparagine.	-	+±.	++	✓

Compare the N-utilization of glycine! (and acetate; glycolic acid!)

Fermentation Tests.

148

March 6, 1948.

EMB:

	Lac	Megal.	Mal	Gal	Glu	Suc	
1 319	-	-	-	±?	-?	-	
2 320	-	-	+	±?	-	-	Growth limited.
3 321	±	-	+	±?	+	-	(108)
4 322	±	-	+	±	+	-	(108) Megal -!
5 323	-	-	+	+	+	-	
6 324	-	-	+	+	+	-	
7 325	-	-	+	+	+	-	
8 326	-	-	+	-	+	-	
9 331	-	-	+	-	+	-	
10 332	-	-	+	+	+	-	
11 333	-	-	+	+	+	-	
12 334	-	-	+	+	+	-	
13 335	-	-	+	+	+	-	

Glu- Megal- ! cf. 108

Mg:

W-329
W-330
W-335.

	TRE 24.	TRE 36 hr.	48.
Tr.	+++	++	✓
W-1	---	±	-
W-60	---	±	-
W-102	+++	+++	-
W-108	-	-	-
W-145	+++	+++	✓ with +++ pop.
W-306	-	-	-
W-327	108 Mal +	-	-
W-328	108 Mal +	-	-
W-117	-	-	-

} Repeat tests
in purported
negative.
Select for specific
reactions.

W-60

March 5, 1948.

Heavily mottled: P7

W-243. Lac +++ * 99%+. Test on Glu, Mal. 60: Mal+. 34 Glu+ No S.R.

Mal -

Glu -

W-145 Me-gal. ++ * Heavily weak+. Test on Lac. 16+. Test on Glu, Mal.

W-125 Me-gal. +++ * All+ Test on Lac. 10+.

W-120 Me-gal. ± ~~++~~ ±

W-45 Me-gal. ± ++ * 41+/lac all+.

P8.

W-117 Tre. P9. +++ * 85%+. Test on maltose 15 all+. Test on ⁺ glu+lac. All+, +.

W-60 Tre. +++ * 60% weak+. Test on maltose. (6-).

Re-test on trehalose: +±. S.O. (1) on sucrose.

† W-117 controls easily distinguished from +'s., and between glu (±) and lac (-).

Papillae from 327, 108 on trehalose tested on glucose.

327: 4+, 2- } Re-test on trehalose. 149-1-6

108: All- } 149-7-10 (11,12 S.O.)

When retested, no distinctive Tre+, unless Glu+, noted.

Test Recombination of C2 mutants.

150

March 6, 1948.

Pupae washed suspensions + plate 1 ml each on lac EMS 'A6.

	A 8.
1. W93	-
2. W138	-
3. W139	-
4. Y87 x W93	-
5. Y87 x W138	-
6. Y87 x W139	-
7. W93 x W138	-
8. W93 x W139	-
9. Y87.	-

No evidence of recombination. Mixed culture must be tried.